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(54) Title: METHOD FOR AUTOMATED, LARGE-SCALE MEASUREMENT OF THE MOLECULAR FLUX RATES OF THE PROTEOME OR THE ORGANEOME USING MASS SPECTROMETRY

(57) Abstract: Disclosed here is a method for measuring the kinetics (i.e., the molecular flux rates - synthesis and breakdown or removal rates) of a plurality of proteins or organic metabolites in living systems. The methods may be accomplished in a high-throughput, large-scale automated manner, by using existing mass spectrometric profiling techniques and art well known in the fields of static proteomics and static organeomics, without the need for additional biochemical preparative steps or analytic/instrumental devices.

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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/23340

## A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12Q 1/02

US CL : 435/29

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/29

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)  
EAST, NPL

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GYGI, S.P. et al. Using mass spectrometry for quantitative proteomics. Proteomics: A friends Guide. July 2000, pages 31-36, see entire document.	1-45
Y	VEENSTRA et al. Proteome Analysis Using Selective Incorporation of Isotopically Labeled Amino Acids. J. Am Soc Mass Spectrom. 2000, Vol. 11, pages 78-82, See entire document.	1-45
Y	ONG et al. Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics. Molecular and Cellular Proteomics. May 2002, Vol. 1, pages 376 - 386. See entire document.	1-45
Y	PAPAGEORGOPOULOS et al. Measuring Protein Synthesis by Mass Isotopomer Distribution Analysis (MIDA). Analytical Biochemistry. 1999, Vol. 267, pages 1-16, see entire document.	1-45
Y	PASA-TOLIC et al. High Throughput Proteome-Wide Precision Measurements of Protein Expression Using Mass Spectrometry. J. Am. Chem. Soc. 1999, Vol. 121, pages 7949-7950. See entire document.	1-45



Further documents are listed in the continuation of Box C.



See patent family annex.

\* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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document member of the same patent family

Date of the actual completion of the international search

22 June 2004 (22.06.2004)

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## INTERNATIONAL SEARCH REPORT

## C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	SHEVCHENKO et al. Rapid 'de Novo' Peptide Sequencing by a Combination of Nanoelectrospray, Isotopic Labeling and a Quadrupole/Time-of-Flight Mass Spectrometer. Rapid Comm. Mass Spectrom. 1997, Vol. 11, pages 1015-1024. See entire document.	1-45
Y	HANSEN et al. A Practical Method for Uniform Isotopic Labeling of Recombinant Proteins in Mammalian Cells. Biochemistry. 29 December 1992. Vol. 31, No. 51, pages 12713-12718, see entire document.	1-45
Y	BIER, D.M. Stable isotopes in biosciences, their measurement and models for amino acid metabolism. Eur J Pediatr. 1997. Vol 156 [Supp 1], pages S2-S8, see entire document.	1-45

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/23340

## Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 46 and 47  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:  
because the claimed inventions are directed to non-statutory subject matter.
3. ☐ Claim Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐  
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.